CellPress

Professor Donald Metcalf (1929–2014)

Donald Metcalf was one of the world's leading experimental hematologists. His discoveries in the fields of colony stimulating factors, cell signaling, and blood cell development not only established many of the concepts of modern hematology but also led to revolutions in the treatment of patients undergoing chemotherapy and bone-marrow transplants, treatments that have benefitted millions of patients worldwide. Much of his scientific work was at the Walter and Eliza Hall Institute in Melbourne during the time it was a hotbed of research on the adaptive immune system. However, he stood apart in insisting on the importance of what we now term the innate immune system and waited for immunologists to catch up, as they now have. Although the last thing he would have wanted is to be termed an immunologist, he nevertheless made major contributions to the field. Almost forgotten but crucial at the time was his early work on the biology of the thymus, an organ whose function had yet to be properly defined. He continued as a collaborator with immunologists, bringing his insights and laboratory skills to problems of immune system development, which, for him, was just another branch of hematology.

Donald Metcalf (Don, to us) was born in the small country town of Mittagong in New South Wales, Australia. He was the middle child of three (sisters Beryl and Rosalind), and both of his parents were schoolteachers. His father, Donald Davidson Metcalf, was the son of a Scottish migrant and no doubt ingrained in his son the virtues of hard work and discipline. Don was constantly on the move as his parents took up posts at various country schools between the times of the Great Depression and World War II. Don was a bright and conscientious student and he obtained a scholarship to study medicine at the University of Sydney. During his degree, Don worked in the laboratory of Professor Patrick de Burgh on the ectromelia virus and this experience had a profound effect on him, committing him to a life of medical research. He graduated in 1953 as a Bachelor of Medicine and Surgery and, during his medical residency at the Royal Prince Alfred Hospital, he met a beautiful young nurse, Josephine Lentaigne, who would become his wife and life-long partner. They would raise four daughters, Katherine, Mary-Ann, Penelope, and Johanna.

In 1954, Don was offered the inaugural Carden Fellowship of the then Anti-Cancer Council of Victoria to work at the Walter and Eliza Hall Institute of Medical Research. This fellowship provided Don with a salary and some money for research. Extraordinarily, Don held this fellowship continuously until his death. The eminent virologist Sir Macfarlane Burnet was Director of the Institute at that time, but he had rather fixed ideas on areas of research and had little time for those who thought that cancer was a disease that could be cured. Accordingly Don was banished to crude laboratories above the animal house. The terms of the Carden Fellowship were to "find the cause and cure of cancer" and in honoring this commitment Don turned to an investigation of the role of the thymus in lymphoid leukemia development. From 1956 to 1958, Don undertook a postdoctoral fellowship at Harvard University with Jacob Furth, whose ideas regarding cancer development as "an imbalance of cell regulators" would significantly influence Don's thinking.

Don returned to the Walter and Eliza Hall Institute and initially continued his work on the thymus, an organ that very few people were interested in at the time. One notable exception was Jacques Miller who would become a close colleague and friend at the Institute. One has only to read through Don's 1966 monograph "The Thymus: Its Role in Immune Responses, Leukaemia Development and Carcinogenesis" to realize the remarkable aptitude he possessed as a researcher. Meticulous in experimental design and execution, he was a peerless anatomist, pathologist, and cytologist. His writing was clear and precise, notable for its separation of observation and experimental fact from theoretical speculation. These were the defining characteristics that Don would carry throughout his scientific career.

Don's analysis of thymic leukemogenesis in the AKR mouse strain was considered definitive. But his studies also provided fundamental information on the functions of the normal thymus. He documented the effects of thymectomy and thymic grafting on peripheral lymphoid tissue and with an elegant series of thymic grafting experiments demonstrated the autonomous control of lymphocyte proliferation within the thymus. He demonstrated that lymphocytes populating grafted thymic tissue were derived from the host and proposed that this occurred from the then speculative hemopoietic stem cell within the host bone marrow. His painstaking radioautographic analysis of thymic cell kinetics demonstrated that the thymus generated about one third of its cell number each day and led him to conclude that most of these thymocytes born in the thymus must die there. Few at the time accepted that a biological system could be that wasteful, so it was many years before extensive intrathymic death was accepted as the necessary price of

positive and negative selection of the T cell repertoire. Don's scrupulous ''balance sheet'' approach to thymocyte kinetics formed the basis of subsequent work on T cell development in the thymus by Ken Shortman and Roland Scollay at the Walter and Eliza Hall Institute.

Don next aimed to discover the regulators controlling blood cell formation, and this theme would possess him for the next 50 years. In 1965, Ray Bradley, a scientific collaborator at the University of Melbourne, walked across the road to show Don dishes of semi-solid agar in which were growing small cellular colonies from mouse bone marrow. These were dependent on an appropriate underlayer of tissue fragments that ''conditioned'' the medium and presumably secreted growth factors. It was this technique that allowed the growth of individual blood cells in vitro for the first time. Both Don and Bradley realized that the growth of these colonies, each derived from a single cell, required the addition of something to the culture medium. These were proposed as soluble 'factors' that supported the survival and clonogenic growth of myeloid bone marrow and spleen blood cell colonies. Don and Bradley termed these ''colony-stimulating factors'' (CSFs). The semi-solid agar culture system had provided a robust method for detecting and quantifying the concentrations of the proposed but yet-undiscovered CSFs. Much would hinge on this astute observation, which was also made contemporaneously by a group headed by Leo Sachs at the Weizmann Institute in Israel. Optimizing this clonogenic culture system laid the groundwork for the purification and genetic cloning of the CSFs, a Herculean task that would take several decades and hundreds of collaborators. Importantly, CSFs would prove to be critical for cells that comprise the innate immune system by regulating their proliferation, differentiation, effector function, and survival.

Don's other great insight was that this culture system provided a method by which he could explore the cellular basis of blood cell production in great detail. This was another theme to which he was committed to for the rest of his career. Together with James Till and Ernest McCulloch at the Ontario Cancer Institute, Don was a pioneer in revealing the hematopoietic hierarchy, beginning at what he regarded as the apex of progenitor cell development, the multipotential blast colony-forming cell.

Don Metcalf led from the front the effort to purify and clone CSFs. Now deputy director of the Walter and Eliza Hall Institute under the new director, Sir Gustav Nossal, Don formed a team across the institute and the Melbourne branch of the Ludwig Institute for Cancer Research. These important collaborators included Nick Nicola, Antony Burgess, and Richard Stanley, who possessed the necessary skills and expertise outside Don's own recognized talents, but carried the same level of commitment to the task. Purification of the CSFs required the development of the then emergent technologies of protein purification by high-performance liquid chromatography to demonstrate the existence of four principal CSFs that supported myeloid colony growth and differentiation. This required two decades of continuous work.

Ultimately, it was amino-acid sequencing and the arrival of molecular biology that allowed the cloning of the murine and human genes for CSFs. It speaks to Don's fastidiousness that he was only convinced a pure CSF had been discovered when they were in possession of the CSF's genetic sequence. The Parkville group obtained extensive amino-acid sequence for murine G-CSF and GM-CSF in collaboration with Lindsay Sparrow from the CSIRO and Richard Simpson at the Ludwig Institute. A further collaboration between Metcalf, Burgess, Nicola, Anne Kelso, Nick Gough, and Ashley Dunn led to the cloning of the murine GM-CSF gene; the G-CSF gene being cloned by Nagata in Japan. It was Don's leadership that ushered in the era of molecular hematology, which made possible the efficiencies of scale required for CSF production for research and clinical applications.

Potential benefits ever at the forefront of his thinking and a key motivation, Don single-mindedly sought to translate his discoveries into benefits for patients with blood cancers. Clinical trials and translational research were well underway and defined clinical uses for CSFs, in particular G-CSF for supporting neutrophil recovery, which is required to replenish the innate immune system following chemotherapy. During these trials performed in collaboration with clinicians Richard Fox, Glenn Begley, and William Sheridan, Don and Uli Dührsen first observed that stem and progenitor cells moved into the peripheral blood following injection of G-CSF. This single unexpected observation led to the paradigm shift of mobilizing stem cells into the blood for collection, largely replacing bone marrow as a stem cell source for transplantation.

Although CSFs can be used as a therapy to boost blood cell production, more recently John Hamilton, Burgess, Ian Wicks, and others, along with Don, recognized that a ''dark side'' to their action could occur in inflammatory disease. Here, M-CSF and GM-CSF have been shown to be pro-inflammatory in collagen induced arthritis through regulation of macrophage- and granulocyte-lineage cells. The pioneering work to identify the structure of CSFs and cell surface receptors for CSFs led by Don, David Gearing, Gough, and Nicola decades ago became instrumental in the development of CSF antagonists that could be used for treating diseases such as rheumatoid arthritis. Building on this fundamental work, programs that have led to the development of therapeutics to target both ligand and receptors in inflammatory diseases have reached phase III clinical trials.

For five decades, Don Metcalf stood at the very center of this maelstrom of scientific and clinical activity. Yet, even in his 80s, he collaborated at the bench with the immunologists Ken Shortman, Shalin Naik, and Priyanka Sathe in their studies on dendritic cell development, using his clonal colony assays to determine the hematopoietic potential of bone-marrow precursor populations. As a consequence this non-immunologist even published in *Immunity*!

Don was known for his formidable work ethic and exacting scientific observations to the point of obsession. He remained at the laboratory bench and beside his beloved microscope for his entire career. Don was brutally honest with a dry wit and had little tolerance for work he considered superficial. Typical were his comments on those hoping to patent SNPs without

knowledge of the genes or their functions: they are like "blind dogs staggering around the room, bumping into chair legs, then pissing on them." But Don was a giving collaborator whose natural qualities of leadership had engendered great respect and indeed great affection from those who worked with him. His colleagues effectively became an extended family.

Don Metcalf was a scientist with rare insight and great foresight. We will miss him dearly.

Ken Shortman,[1,3]
Nicos A. Nicola,[2,3]
and Ashley P. Ng[2,3,*]
[1]Division of Immunology, The Walter And Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia
[2]Division of Cancer and Haematology, The Walter And Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia
[3]Department of Medical Biology, University of Melbourne, Parkville, Victoria 3010, Australia
*Correspondence: ang@wehi.edu.au
http://dx.doi.org/10.1016/j.immuni.2015.01.003